Figure 1

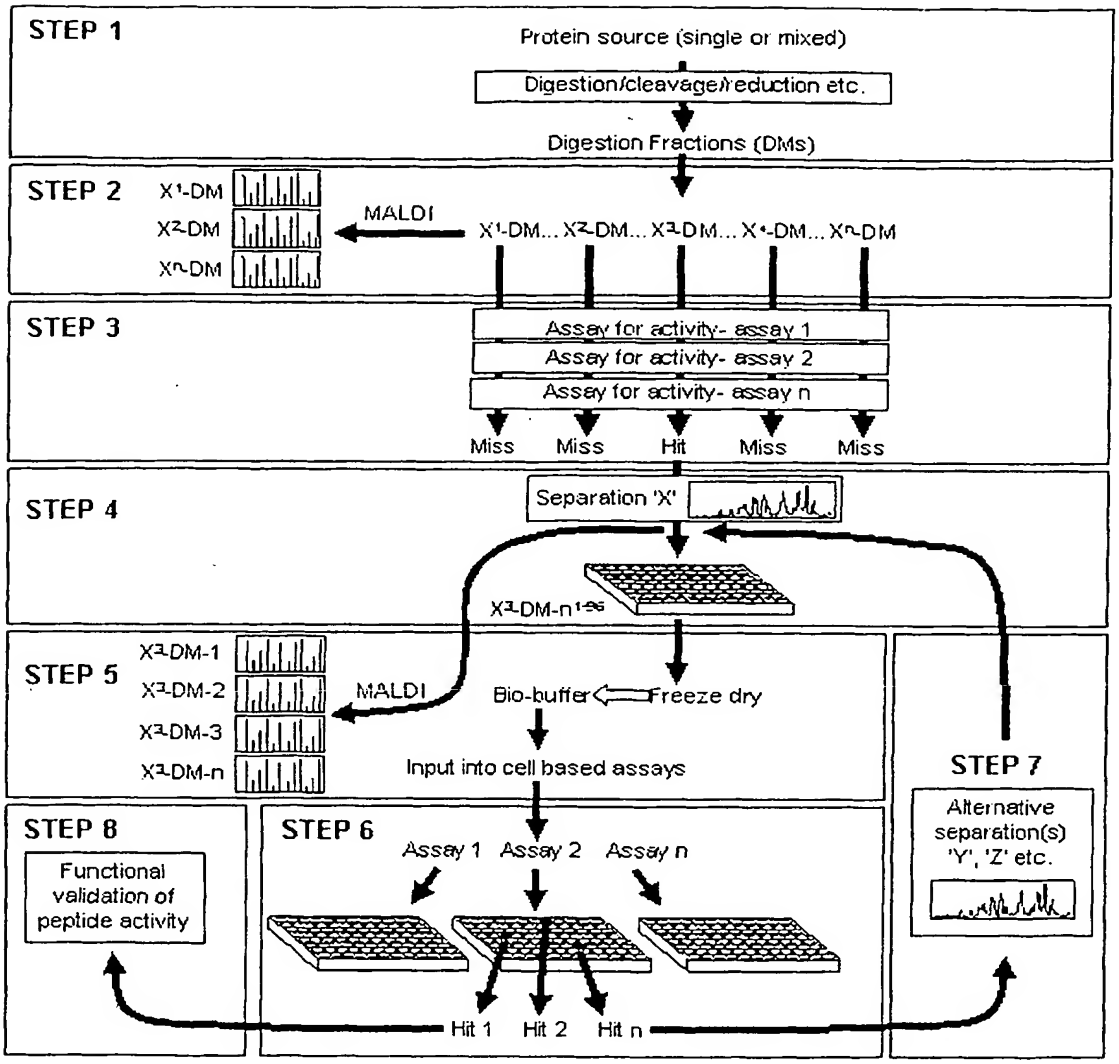

STEP 1
Protein source (single or mixed)
Digestion/cleavage/reduction etc.
Digestion Fractions (DMs)
STEP 2
X1-DM
X2-DM
Xn-DM
MALDI
X1-DM... X2-DM... X3-DM... X4-DM... Xn-DM
STEP 3
Assay for activity- assay 1
Assay for activity- assay 2
Assay for activity- assay n
Miss Miss Hit Miss Miss
STEP 4
Separation 'X'
X3-DM-n1-96
STEP 5
X3-DM-1
X3-DM-2
X3-DM-3
X3-DM-n
MALDI
Bio-buffer
Freeze dry
Input into cell based assays
STEP 7
Alternative separation(s) 'Y', 'Z' etc.
STEP 8
Functional validation of peptide activity
STEP 6
Assay 1 Assay 2 Assay n
Hit 1 Hit 2 Hit n

Figure 2

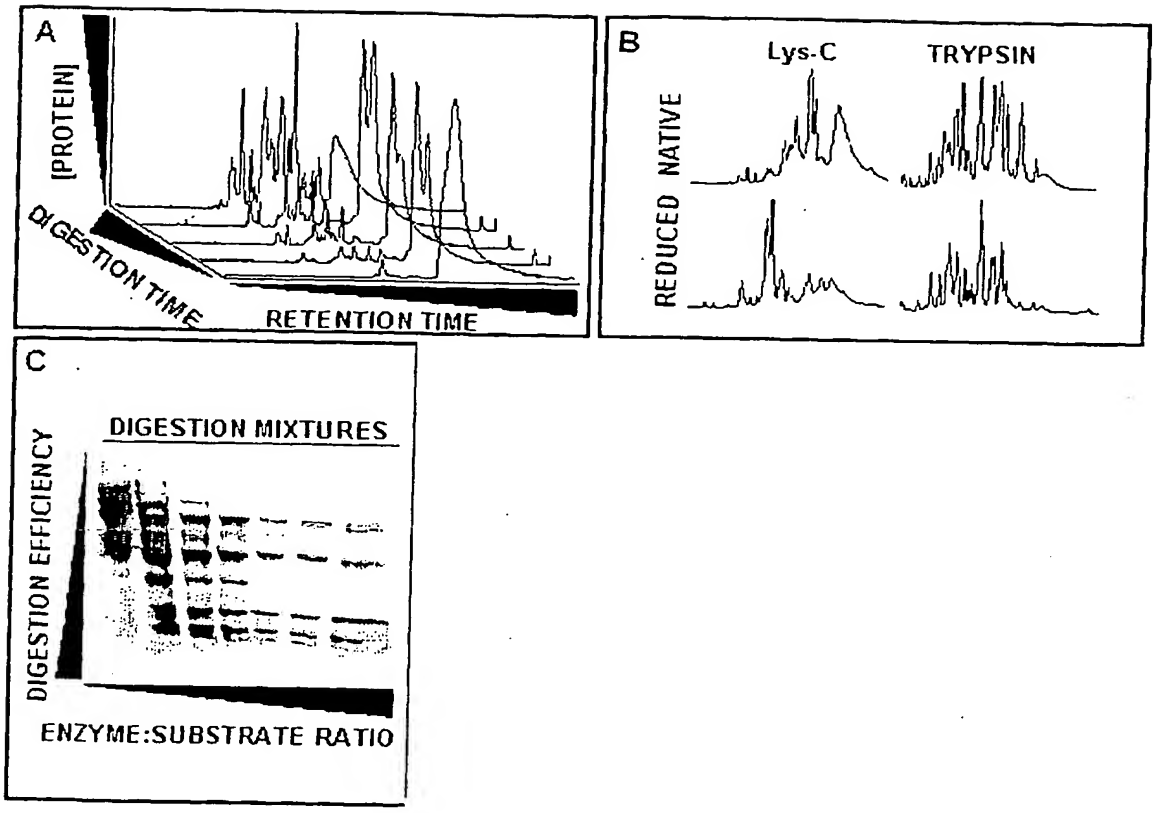
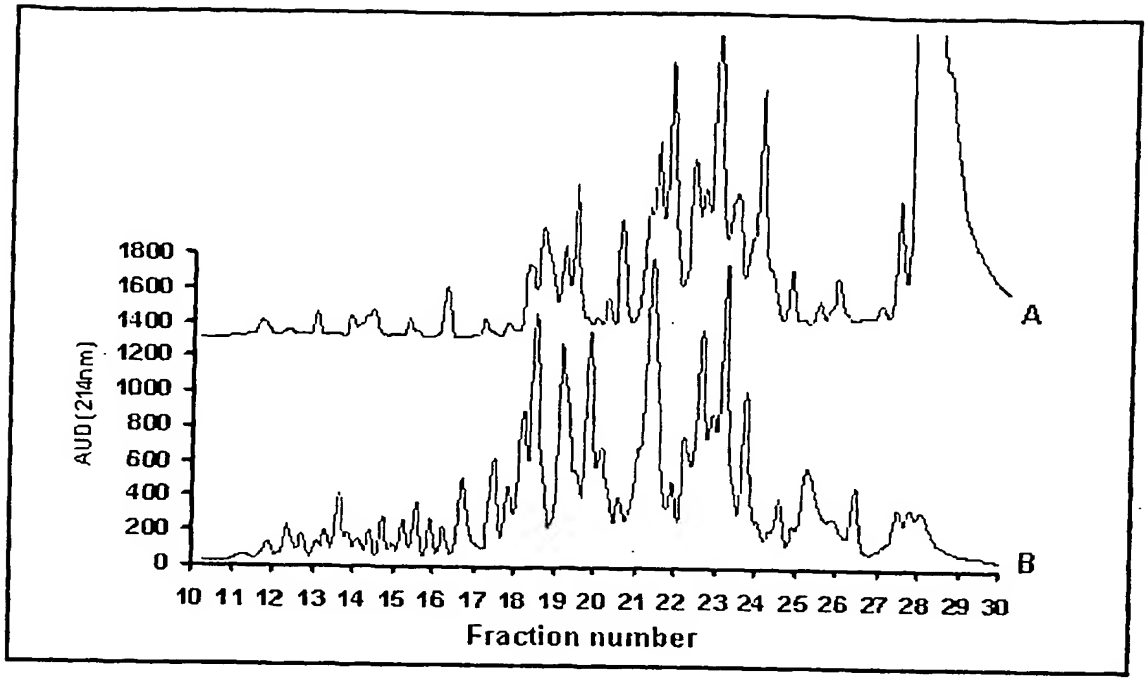

A
[PROTEIN]
DIGESTION TIME
RETENTION TIME
B
Lys-C
TRYPSIN
REDUCED
NATIVE
C
DIGESTION MIXTURES
DIGESTION EFFICIENCY
ENZYME:SUBSTRATE RATIO

Figure 3


AUD (214nm)
1800
1600
1400
1200
1000
800
600
400
200
0
10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Fraction number
A
B

10/520624

Figure 4

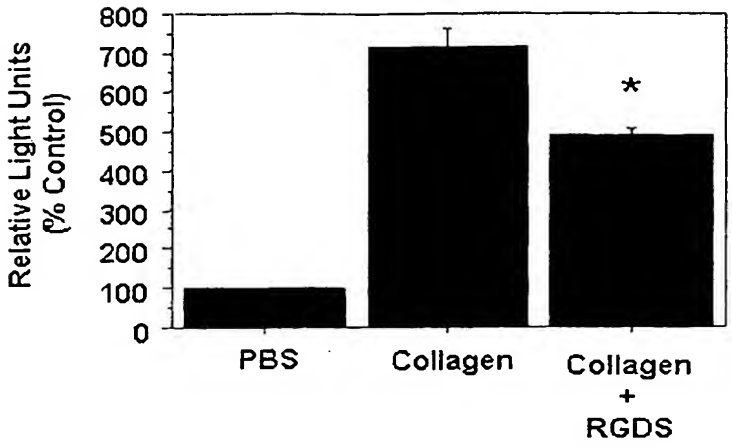


Figure 5

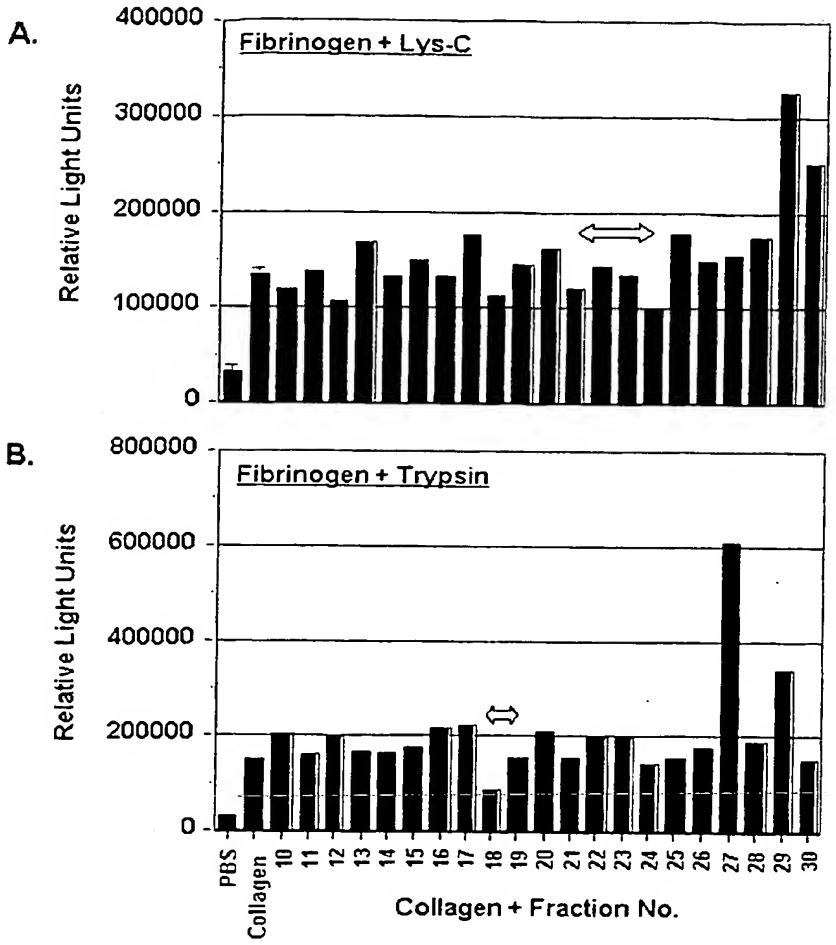

A.
Fibrinogen + Lys-C
Relative Light Units
400000
300000
200000
100000
0
B.
Fibrinogen + Trypsin
Relative Light Units
800000
600000
400000
200000
0
PBS
Collagen
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
Collagen + Fraction No.

Figure 6

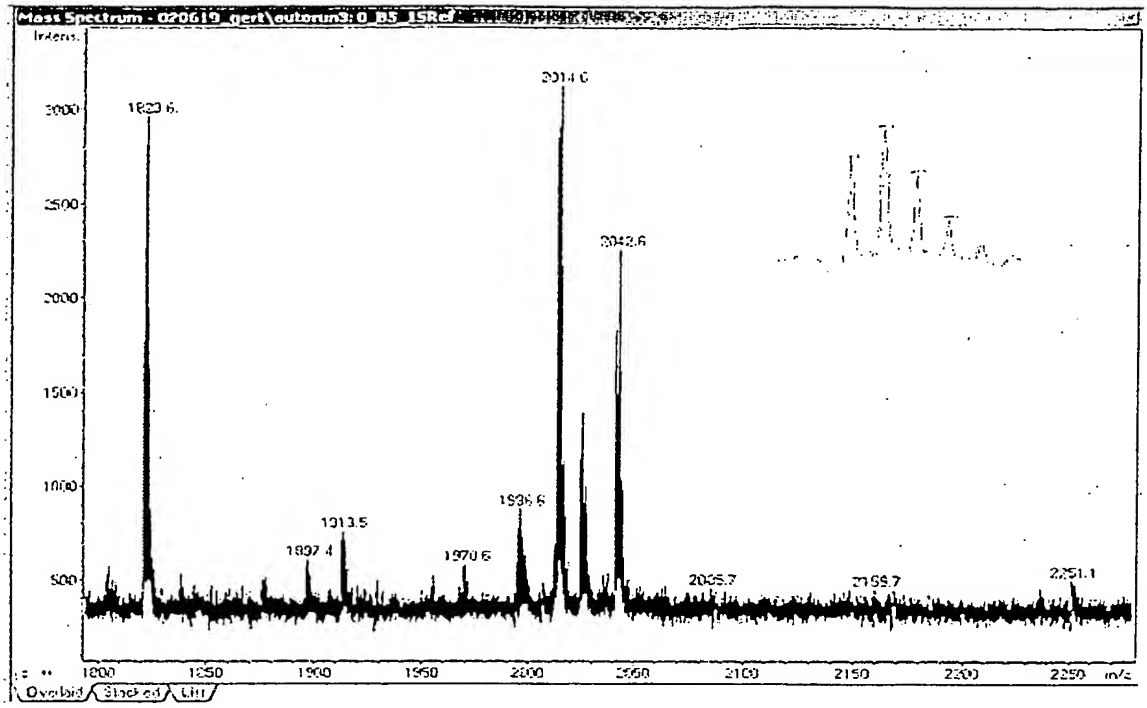

Mass Spectrum - 020619_gert\autorun3: 0_B5_1Ref
Intens.
3000
2500
2000
1500
1000
500
1823.6
1897.4
1913.5
1970.6
1936.6
2014.6
2048.6
2085.7
2159.7
2251.1
1800
1850
1900
1950
2000
2050
2100
2150
2200
2250
m/z
Overlaid
Stacked
List

Figure 7

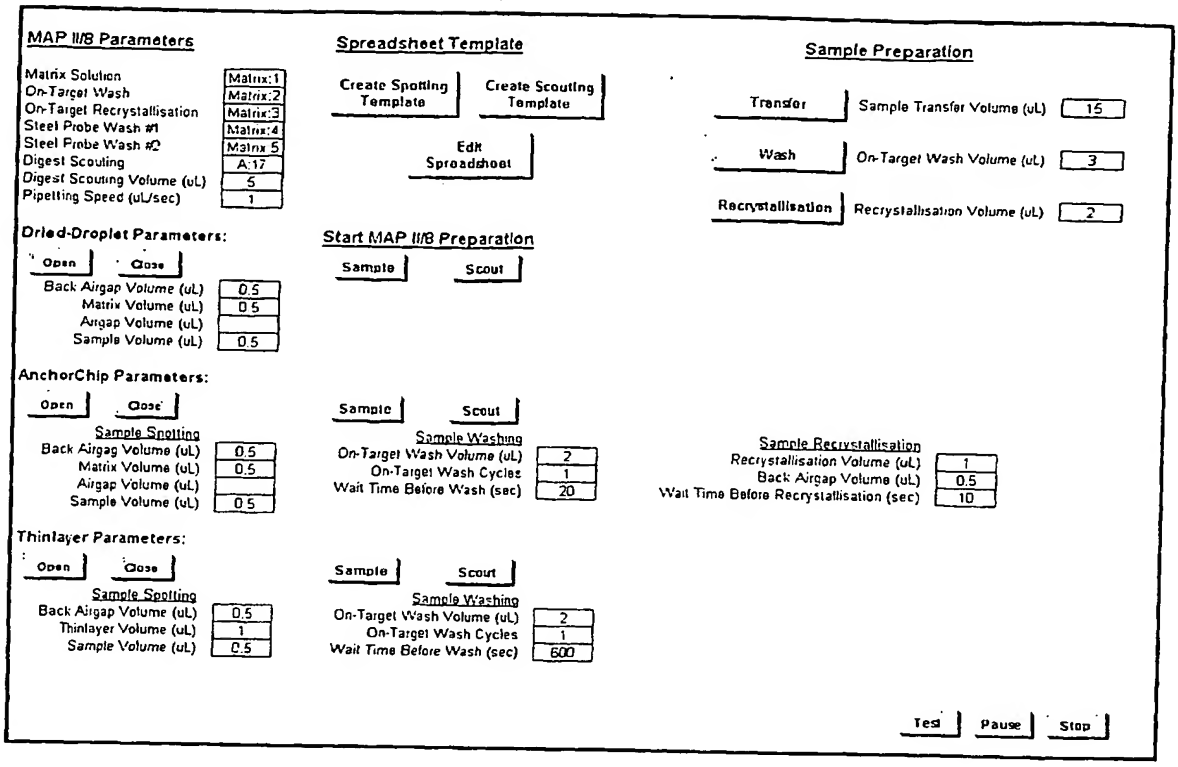
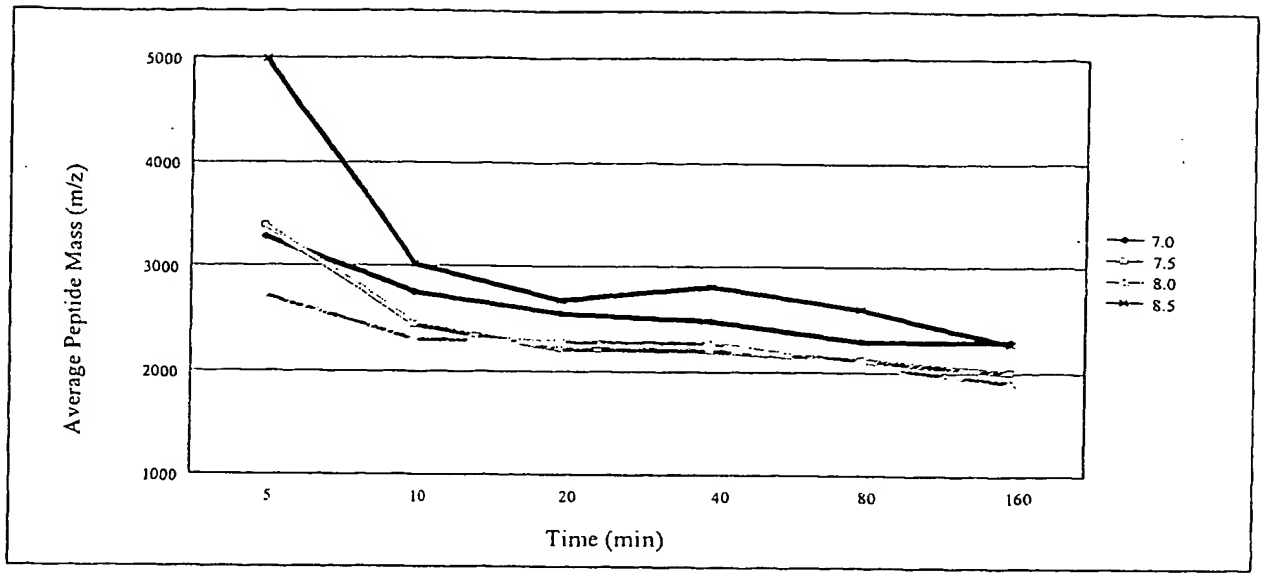

MAP II/8 Parameters
Matrix Solution
On-Target Wash
On-Target Recrystallisation
Steel Probe Wash #1
Steel Probe Wash #2
Digest Scouting
Digest Scouting Volume (uL)
Pipetting Speed (uL/sec)
Matrix:1
Matrix:2
Matrix:3
Matrix:4
Matrix:5
A:17
5
1
Spreadsheet Template
Create Spotting Template
Create Scouting Template
Edit Spreadsheet
Sample Preparation
Transfer
Sample Transfer Volume (uL)
15
Wash
On-Target Wash Volume (uL)
3
Recrystallisation
Recrystallisation Volume (uL)
2
Dried-Droplet Parameters:
Open
Close
Back Airgap Volume (uL)
Matrix Volume (uL)
Airgap Volume (uL)
Sample Volume (uL)
0.5
0.5
0.5
Start MAP II/8 Preparation
Sample
Scout
AnchorChip Parameters:
Open
Close
Sample Spotting
Back Airgap Volume (uL)
Matrix Volume (uL)
Airgap Volume (uL)
Sample Volume (uL)
0.5
0.5
0.5
Sample
Scout
Sample Washing
On-Target Wash Volume (uL)
On-Target Wash Cycles
Wait Time Before Wash (sec)
2
1
20
Sample Recrystallisation
Recrystallisation Volume (uL)
Back Airgap Volume (uL)
Wait Time Before Recrystallisation (sec)
1
0.5
10
Thinlayer Parameters:
Open
Close
Sample Spotting
Back Airgap Volume (uL)
Thinlayer Volume (uL)
Sample Volume (uL)
0.5
1
0.5
Sample
Scout
Sample Washing
On-Target Wash Volume (uL)
On-Target Wash Cycles
Wait Time Before Wash (sec)
2
1
600
Test
Pause
Stop

Figure 8


Average Peptide Mass (m/z)
5000
4000
3000
2000
1000
5
10
20
40
80
160
Time (min)
7.0
7.5
8.0
8.5